

Supplementary Material

***Acinetobacter baumannii*: epidemiological and β-lactamase data from two tertiary academic hospitals in Tshwane, South Africa**

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Table S1: Primer nucleotide sequences of antimicrobial resistance genes in clinical *A. baumannii* isolates

Target	Primer name	Primer sequence (5' to 3') [#]	Amplicon size (bp)	Primer concentration used (μM)	Reference	
Multiplex I:						
CTX-M	CTX-M-U (F)	ATGTGCAGYACCAAGTAARGTKATGGC	593	0.25	Monstein <i>et al.</i> , 2007	
	CTX-M-U (R)	TGGGTRAARTARGTSACCAGAACAGCGG				
SHV	blashv (F)	AGCCGCTTGAGCAAATTAAAC	713	0.2	Dallenne <i>et al.</i> , 2010	
	blaSHV (R)	ATCCCAGATAAAATCACCAC				
TEM	TEM (F)	TGCCGCATAACTATTCTCAGAATGA	445	0.3	Monstein <i>et al.</i> , 2007	
	TEM (R)	ACGCTCACCGGCCTCCAGATTAT				
Multiplex II:						
IMP	blaIMP (F)	TTGACACTCCATTACDG ^{**}	139	0.2	Dallenne <i>et al.</i> , 2010	
	blaIMP (R)	GATYGAGAATTAAAGCCACYCT				
KPC	blaKPC (F)	CATTCAAGGGCTTCTGCTGC	538	0.2		
	blaKPC (R)	ACGACGGCATAGTCATTGTC				
VIM	blaVIM (F)	GATGGTGTGGTCGCATA	390	0.5		
	blaVIM (R)	CGAATGCGCAGCACCAG				
Multiplex III:						
GES	blages (F)	AGTCGGCTAGACCCGAAAG	399	0.3	Dallenne <i>et al.</i> , 2010	
	blages (R)	TTTGTCCGTGCTCAGGAT				
PER	blaper (F)	GCTCCGATAATGAAAGCGT	520	0.3		
	blaper (R)	TTCGGCTTGACTCGGCTGA				
VEB	blavEB (F)	CATTCCCGATGCAAAGCGT	648	0.3		
	blavEB (R)	CGAAGTTCTTGGACTCTG				
Multiplex IV:						
GIM	blaGIM (F)	CGTTGCCAGCTTAGCTCAGG	279	0.4	Voets <i>et al.</i> , 2011	
	blaGIM (R)	GCAACTTGATAACCAGCAGTGCG				
NDM-1	blaNDM-1(F)	CCCGGCCACACCAGTGACA	129	0.7		
	blaNDM-1(R)	GTAGTGCTCAGTGTGGCAT				
SIM-1	blasIM (F)	TTGCGGAAGAAGCCAGCCAG	613	0.4		
	blasIM (R)	GCGTCTCCGATTCACTGTGGC				
SPM	blasPM (F)	GGGTGGCTAAGACTATGAAGCC	447	1.3		
	blasPM (R)	GCCGCCAGCTGAATCGG				
Multiplex V:						
OXA-23-like	blaOXA-23 (F)	GATCGGATTGGAGAACAGA	501	0.2	Woodford <i>et al.</i> , 2006	
	blaOXA-23 (R)	ATTCCTGACCGCATTCCAT				
OXA-48	blaOXA-48 (F)	GCGTGGTTAAGGATGAACAC	438	0.2		
	blaOXA-48 (R)	CATCAAGTTCAACCCAACCG				
OXA-51-like	blaOXA-51 (F)	TAATGCTTGATCGGCCTTG	353	0.2		
	blaOXA-51 (R)	TGGATTGCACCTCATCTGG				
OXA-58-like	blaOXA-58 (F)	AAGTATTGGGGCTGTGCTG	599	0.2		
	blaOXA-58 (R)	CCCCTCTGCGCTACATAC				

**Y=T or C; D=A or G or T; [#] All oligonucleotides were synthesised and purified by Inqaba Biotechnical Industries, Pretoria, South Africa

Positive controls:

The positive controls used included three ATCC cultures, namely *A. baumannii* ATCC BAA-1605 (*blaOXA-23* and *blaOXA-51*); *K. pneumoniae* ATCC 8303 [*Klebsiella pneumoniae* carbapenemase (KPC) positive control] and *K. pneumoniae* ATCC BAA-2146 [New Delhi metallo-β-lactamase (NDM) positive control] as well as local clinical isolates that had been identified and sequenced in former departmental studies. The local clinical isolates used included positive controls for the following genes: Cefotaximase-Munich (CTX-M); Guyana extended-spectrum β-lactamase (GES); German imipenemase (GIM); Imipenem metallo-β-lactamase (IMP); *Pseudomonas* extended resistance (PER); Sulphydryl variant (SHV); Seoul imipenem metallo-β-lactamase (SIM); Sao Paulo metallo-β-lactamase (SPM); Temoneira (TEM); Vietnam extended-spectrum β-lactamase (VEB) and Verona integrin-encoded metallo-β-lactamase (VIM).

Cycling conditions for multiplex I to V:

An initial denaturation step of 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, an annealing temperature dependent on the melting temperature of the primer pair (Multiplex I and IV 60°C; Multiplex II 55 °C; Multiplex III and V 56°C) and extension at 72°C for 90 sec, followed by the final extension step at 70°C for 10 min. Positive and negative controls were included in all the M-PCR assays.

Cycling conditions for ISAbal:

The cycling conditions were followed as described by Segal *et al.* (2005) with one modification; an annealing temperature of 52°C was used.

References:

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